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MORPHOLOGIC CHANGES IN THE LYMPHOCYTES OF PERSONS EXPOSED TO IONIZING RADIATIONS

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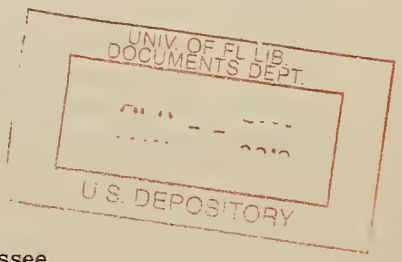

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MORPHOLOGIC CHANGES IN THE LYMPHOCYTES OF PERSONS EXPOSED TO IONIZING RADIATIONS

By Annamae Dickie and Louis H. Hempelmann, M.D.

Examination of supravital preparations of the blood of persons exposed to ionizing radiations and toxic chemicals at the Los Alamos Scientific Laboratory has revealed the presence of an unusually large number of refractive neutral red bodies in the cytoplasm of the circulating lymphocytes. These bodies are also present in smaller numbers in lymphocytes of unexposed persons. They can be distinguished from intracellular vacuoles which also stain with neutral red. The increase in neutral red bodies is evident even when exposure to radiation is within the tolerance range, i.e., not greater than 0.1 roentgen of x-rays or gamma rays per day delivered to the entire body. The neutral red bodies are found in increased numbers after acute exposure to doses of ionizing radiation which do not affect the total white blood cell count, the total lymphocyte count, or the differential blood cell picture.

METHODS AND MATERIALS

This study covers a two-year period from 1944 to 1946. All of the subjects have been employed at the Los Alamos Scientific Laboratory. Except for those in the control group, all persons have been engaged in work which has resulted in exposure to ionizing radiation or toxic chemicals. With rare exceptions, the exposure was limited to one type of ionizing radiation or to one kind of chemical. The subjects included in this report were carefully selected for uniformity of sex and age, and the conditions under which the study was carried out were kept as uniform as possible. The subjects were healthy adult males, the great majority of which were between twenty and thirty years of age. All were in residence at Los Alamos project. All subjects were essentially of the same economic status. Each blood sample was taken in the morning between 8:30 and 10:00 o'clock. Excluded from this series was any subject showing evidence of illness (except for a group of ambulatory controls specifically chosen for upper respiratory infections), extreme fatigue, or blood abnormalities of known or unknown etiology (except radiation).

As far as can be ascertained, dissimilarities between the control and exposed subjects were limited to occupation and number of blood counts per subject. All exposed persons were engaged in laboratory work of some sort, while the occupations of the control group ranged from physical work performed out of doors to clerical work. Relatively few persons in the control series worked under conditions comparable to those under which the exposed subjects worked. As far as the number of blood counts per subject is concerned, not more than one or at the most two blood counts were obtained for each control, while monthly or bimonthly blood counts were done on the exposed personnel. In many cases, this was the only blood count which a control subject had experienced. The emotional response in some of these individuals cannot be ruled out as a factor which may have modified their blood pictures.

Every practical means was used to minimize technical errors and to reduce the statistical variation of individual counts. All pipettes and hemocytometer chambers used in this study were standardized by the National Bureau of Standards. The red blood cell count was determined by examining two sides of a hemocytometer chamber filled with well-mixed diluted blood from a single pipette. Both sides of two chambers containing mixed diluted blood from two pipettes were counted to determine the white blood cell count. The hemoglobin content of the blood was estimated by means of the oxyhemoglobin method of Evelyn. The differential leucocyte picture was obtained by examining three hundred cells - two hundred on a dried film of blood stained with Wright's stain and one hundred in supravital preparations. In most cases, the number of refractive neutral red bodies in 50 lymphocytes was counted and recorded.

Since the morphologic change in the lymphocytes reported in this article can be visualized only in supravital preparations, a detailed account of this technique as used in the Los Alamos Medical Laboratory will be given.

Microscopic slides and cover slips are carefully cleaned with potassium dichromate-sulphuric acid solution. They are then rinsed with distilled water and ethyl alcohol and stored in a dust-free box. A small drop of blood from a fresh stab incision on the finger or lobe of the ear is picked up with a clean cover slip (No. 00). The glass is inverted and allowed to fall on a microscopic slide on which an alcoholic solution of neutral red and Janus green has dried. When the blood has spread between the two surfaces to form a thin film, the cover slip is rimmed with petroleum jelly. The preparation is kept in an ice box until just before examination, when it is allowed to come to room temperature.

In order to obtain adequate uptake of the dyes by the leucocytes, it has been necessary to use much stronger solutions of stain than those recommended in the literature.[1] The dye solution used in this laboratory consists of 40 cc of a saturated alcoholic solution of Janus green and 16.4 cc of a saturated alcoholic solution of neutral red dissolved in 100 cc of absolute ethyl alcohol freshly prepared by the sodium phthalate method. The alcoholic solution is allowed to flow onto clean microscopic slides, and the excess is wiped off immediately. The slides are dried and stored in light-proof, dust-free containers. Freshly stained slides are prepared every 48 hours. Presumably, the intensity of scattered sunlight at this altitude (7300 feet above sea level) modifies the dyes in an unknown manner which makes necessary the use of higher concentrations of staining solutions. The appearance of the cells and uptake of dye by leucocytes resulting from the use of slides prepared with these strong solutions do not differ in any way from that observed in other laboratories where more dilute solutions of supravital dyes can be employed.

RESULTS

It was observed that the lymphocytes of persons exposed to ionizing radiations and toxic chemicals contained more refractive neutral red bodies than did the cells in the blood of unexposed subjects. These bodies are irregularly placed throughout the cytoplasm. They are irregular in shape, vary considerably in size, and have a more decided brick-red color than do the neutral red-staining vacuoles (Figures 1 and 2). There is no increase in number or size on standing twelve hours at ice-box temperature. The number of refractive bodies per cell varies from zero to more than thirty. The refractiveness of these bodies resembles that of the granules in cells of the granulocytic series and distinguishes them from vacuoles and nonrefractive bodies which also stain with neutral red dye.

Examination of the same blood samples stained with Wright's, Giemsa and peroxidose stains revealed no morphologic abnormalities of the lymphocytes similar to that described above.

By using contrast microscopy, additional information can be gained about the structure of the refractive neutral red bodies. If individual cells in a supravital preparation are examined first through an ordinary microscope and then through a phase microscope, it is observed that the refractive neutral red bodies show a high phase contrast while the nonrefractive and vacuolar neutral red staining bodies show a low phase contrast. This indicates that the density of the neutral red bodies is high and that they probably exist in the solid or granular phase. The mitochondria is the only other structure in the lymphocytes that shows a high phase contrast. These bodies are not easily confused with the neutral red bodies under the contrast microscope, as they are smaller, and their green color is poorly transmitted through the phase optical system, while the red color of the refractive bodies is well transmitted.

The increase of the neutral red bodies in the lymphocytes of the exposed groups represents essentially a quantitative rather than a qualitative change. The modification in the number and distribution of neutral red bodies in lymphocytes for subjects variously exposed to radiation is shown in Figure 3. In order to facilitate the demonstration of lymphocytic changes of this nature, it was decided to differentiate between cells which contain an abnormally large number of refractive neutral red bodies and those which do not. Therefore, it was agreed, on a completely arbitrary basis, to designate any lymphocyte with five or less neutral red bodies as "normal" and to consider any cell with six or more bodies as "abnormal". This terminology will be used in the remainder of this article.

One thousand and sixty four hematologic studies on 364 subjects have been analyzed. Figure 5 is a scatter diagram showing the per cent of abnormal lymphocytes in all subjects, divided into control and exposure groups. The explanation of the type and degree of exposure of each group is given in

Figure 1. Microphotograph (1200 x) of supravital preparation of blood cells from a subject recently exposed to small doses of gamma radiation. Arrows point to the two refractile neutral red bodies in the cytoplasm of the lymphocyte in the center of the field. The other cytoplasmic shadows are mitachondria.

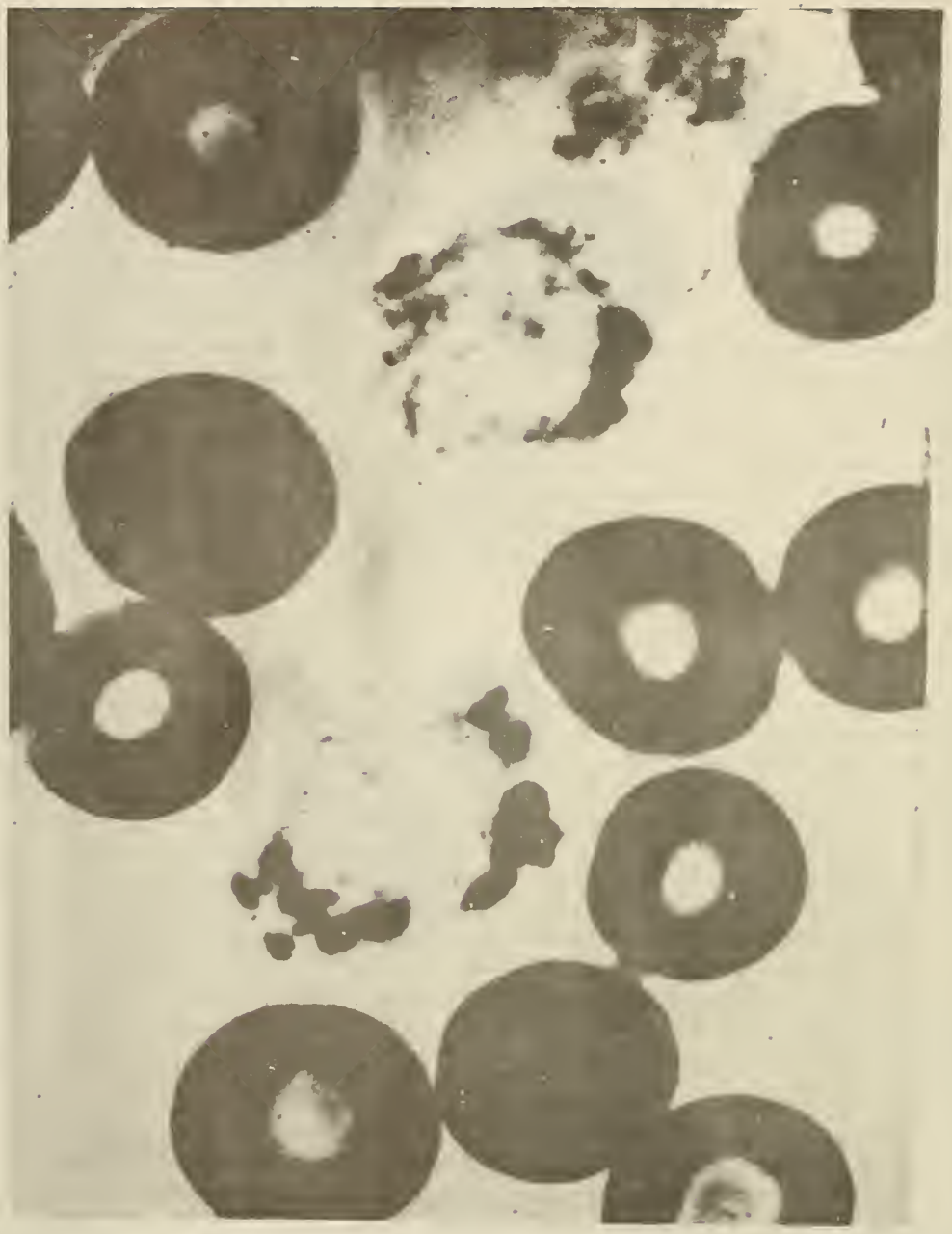

Figure 2. Microphotograph (1200 x) of a supravital preparation of blood from a subject recently exposed to small doses of gamma radiation for a period of two months. There are two lymphocytes in this field. The upper one has three large refractile neutral red bodies. The lower lymphocyte has fifteen of these bodies, some of which are out of focus in this photograph. The large shadow at 3:00 o'clock in the lower cell is formed by two neutral red bodies. The other shadows in the cytoplasm of both cells are mitachondria which stain with Janus green.

Chart 1. This exposure refers to the period 1943 to 1946 and does not take into consideration the previous exposure record of the subject. In practically all cases except those in Radiation Groups II and III, previous exposure to toxic chemicals or radiation is negligible. Each point in the scatter diagram represents the per cent of abnormal lymphocytes for a single subject. In the case of most of the controls, this point is based on one examination; in the case of the exposed subjects, the point represents the average of many (in one instance, thirty) examinations. Where radiation exposure was intermittent, it is impossible to correlate the time of the hematologic examinations with the time of the exposure. For purposes of comparison, Figures 4, 6, 7, and 8 show scatter diagrams of the total white blood count, the total abnormal lymphocyte count, the total lymphocyte count, and the per cent lymphocytes in the differential white blood cell count for each exposure group. As in the case of Figure 5, each point represents an average figure for all examinations on a single individual.

Figures 9 and 10 show the increase in per cent abnormal lymphocytes in persons accidentally exposed to single large instantaneous bursts of general body radiation. It is unfortunate that previous determinations of per cent abnormal lymphocytes had not been made on these subjects. A complete hematologic report of these cases will be presented in a forthcoming article. Single exposures to general body gamma radiation which did not exceed 5 roentgens and which were delivered over a period of several hours produced no increase in the percentage of abnormal lymphocytes.

Similar increases in abnormal lymphocytes have been observed in rabbits and cows exposed to large doses of ionizing radiation. Morphologic changes of this type in the lymphocytes of mice and rats exposed to radiation could not be detected because the large amounts of neutral red dye normally taken up by the cytoplasm of the lymphocytes interferes with the identification of the refractive bodies.

Discussion

A not-too-thorough survey of the literature has failed to disclose previous descriptions of similar morphologic changes in lymphocytes after in vivo exposure to small repeated doses of ionizing radiation. Morphologic changes in living cells of various kinds have been reported following exposure to single large doses of radiation. Prigosen[2] has reported the appearance of neutral red bodies in irradiated tumor cells. Recently, Shrek[3] described an increase in cytoplasmic vacuoles in dark field preparations of lymphocytes after in vitro and in vivo exposure.

Inspection of the scatter diagrams in this report indicates that there is an increase in abnormal lymphocytes in the persons exposed to ionizing radiation. The per cent and total number of abnormal cells can be correlated with the magnitude of chronic exposure. The significance of the data presented in these diagrams is evident without further statistical treatment. There are several features of these diagrams, however, which require further discussion.

A significant difference between controls (Group A, Class I) and the groups exposed to radiation (Group B, Classes I, II, III, and IV) is observed only for total leucocyte count, the proportion and absolute numbers of abnormal lymphocytes. In the case of the total white blood cell count, the difference is statistical and can be demonstrated best by comparing the average counts for each group. Since most of the total counts of all groups lie between 5000 and 9000 cells per cubic millimeter, the value of any individual count has little significance in determining the exposure group into which the subject falls. On the other hand, the difference in the per cent abnormal cells and absolute abnormal cell counts between control and exposed subjects is reflected in the value for an individual subject. Most of the controls have less than 20 per cent abnormal cells or 400 abnormal cells per cubic millimeter, while all of the more consistently exposed subjects show percentages and total numbers of abnormal cells above these levels. There is so little overlap of points for exposed and control groups that the appearance of few or many abnormal cells has more than chance significance in determining the exposure of any given individual.

A difference in percentage and absolute numbers of abnormal lymphocytes also exists between controls and groups exposed to certain chemicals. In this respect, it should be emphasized that the biological action of natural uranium is due to its chemical rather than its radioactive properties. Thus the increase in per cent abnormal lymphocytes in group D is probably related to the chemical effect of uranium. The increase in per cent abnormal cells in Group A, Class II (Figure 5) is added evidence

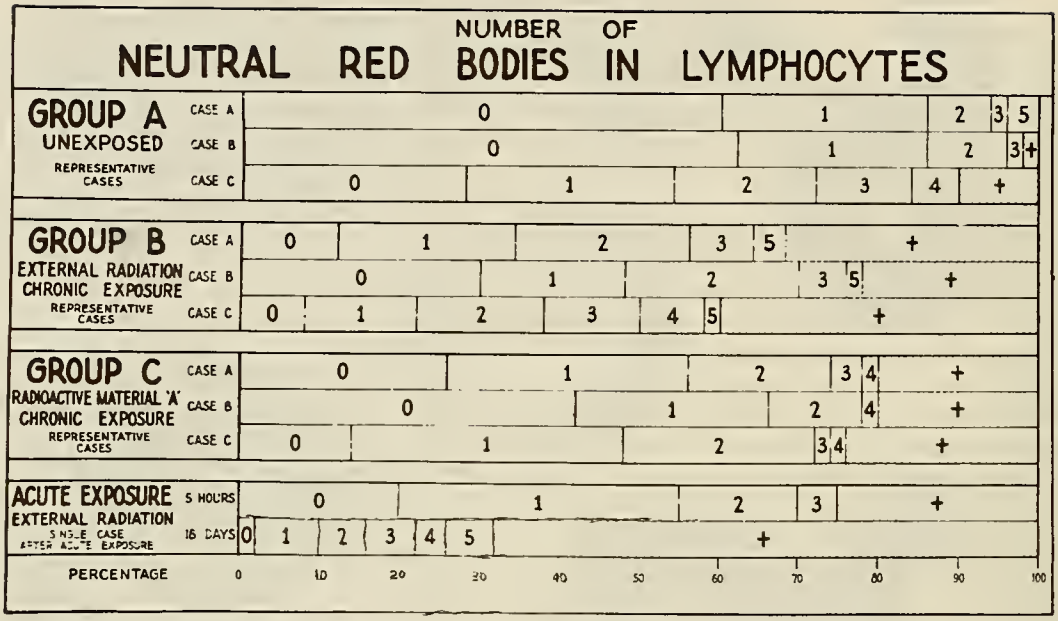

Figure 3. This chart arranges the lymphocytes of representative individuals in various exposure groups according to the number of refractive bodies which they contain. The bands extending across the chart represent 100 per cent of the lymphocytes. The scale of lymphocytes in per cent is shown at the bottom of the chart. The numerals in each segment of the band denote the number of refractive bodies in that fraction of the lymphocytes. The lymphocytes containing more than five bodies are lumped together in one group designated by the plus sign. Thus, in Case A Group A, 60 per cent of the lymphocytes contained no neutral red bodies; 25 per cent, one body; 8 per cent, two bodies; 2 per cent, three bodies; and 5 per cent, five bodies.

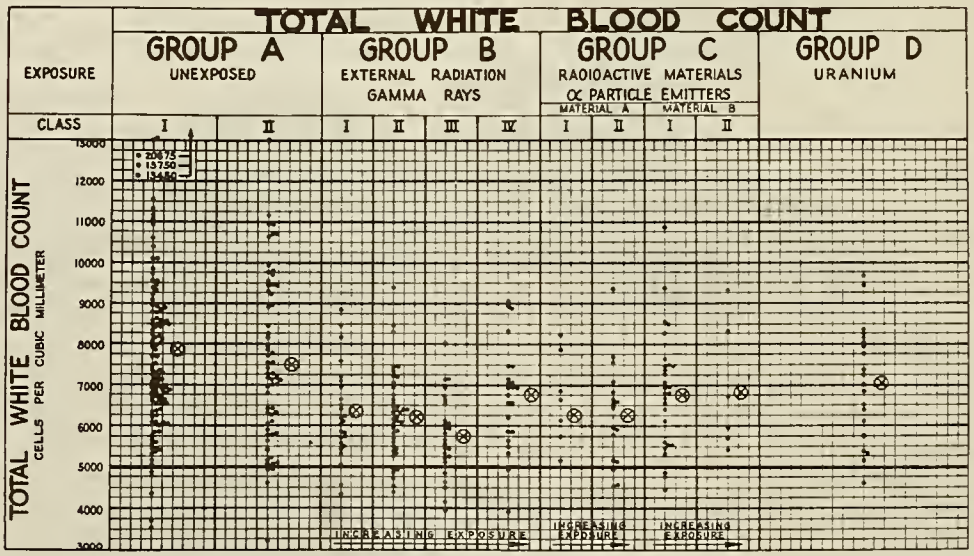

Figure 4. Scatter diagram showing the total leucocyte count for individuals in each exposure group. The cross within the circle represents the average leucocyte count for the group.

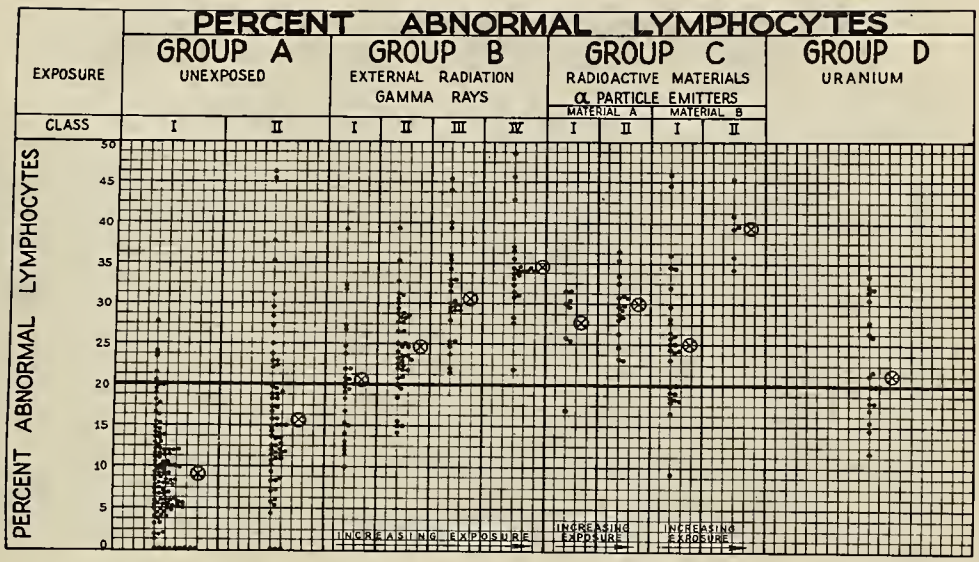

Figure 5. Scatter diagram showing the per cent abnormal lymphocytes for persons in each exposure group. The cross within the circle indicates the average value for the group.

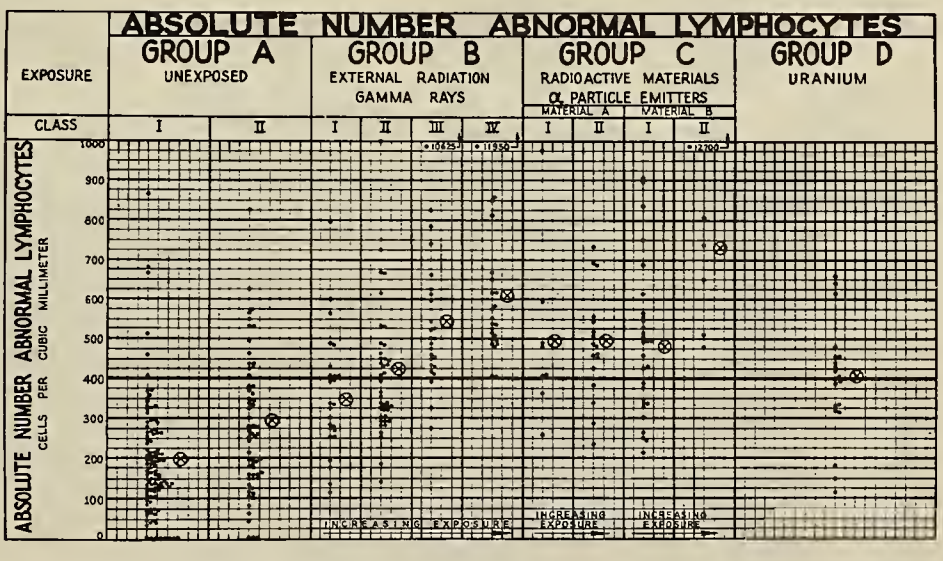

Figure 6. Scatter diagram showing the total abnormal lymphocytes per cubic millimeter for individuals in each exposure group. The cross within the circle indicates the average for the group.

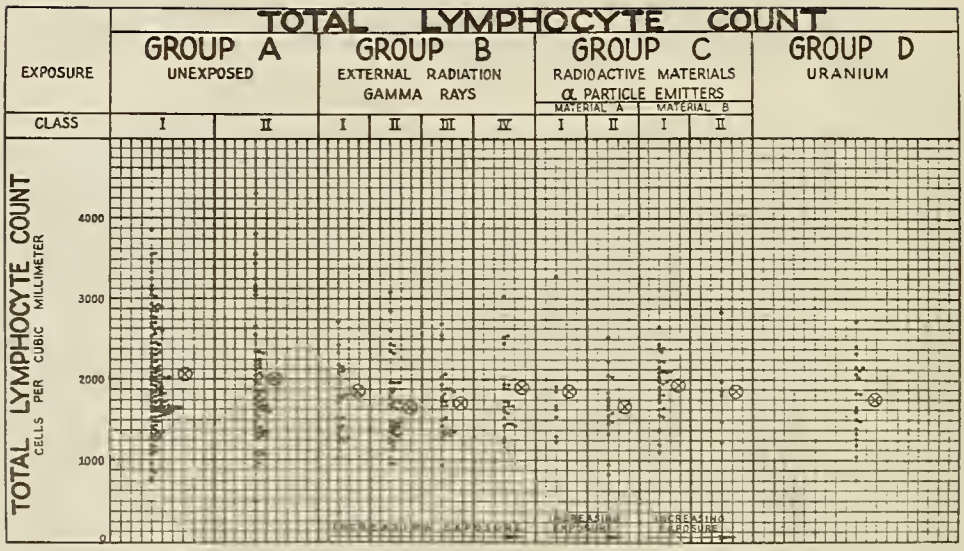

Figure 7. Scatter diagram showing the total lymphocyte count for persons in each exposure group. The cross within the circle indicates the average for the group.

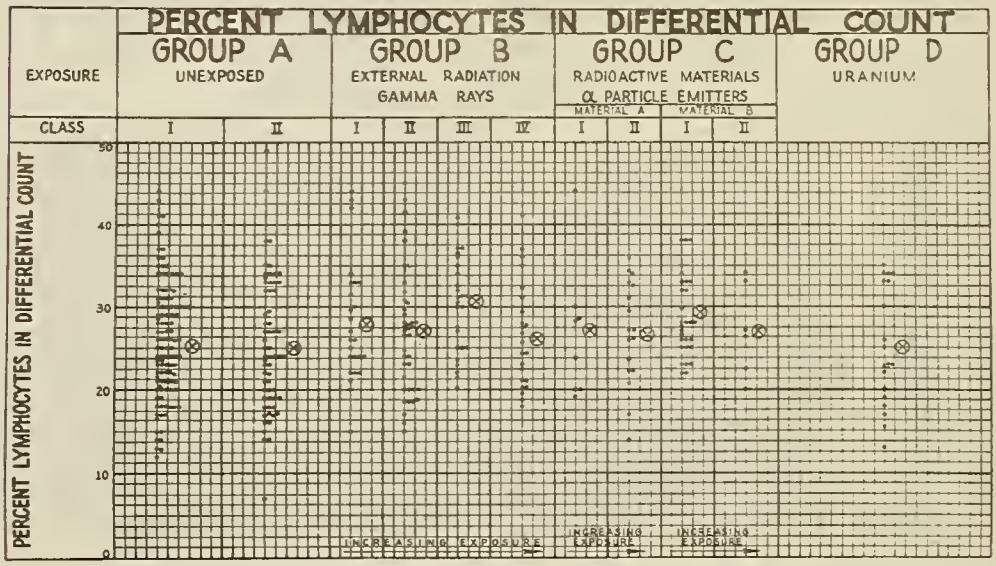

Figure 8. Scatter diagram showing the per cent lymphocytes in the differential count of individuals in each exposure group. The cross within the circle indicates the average for the group.

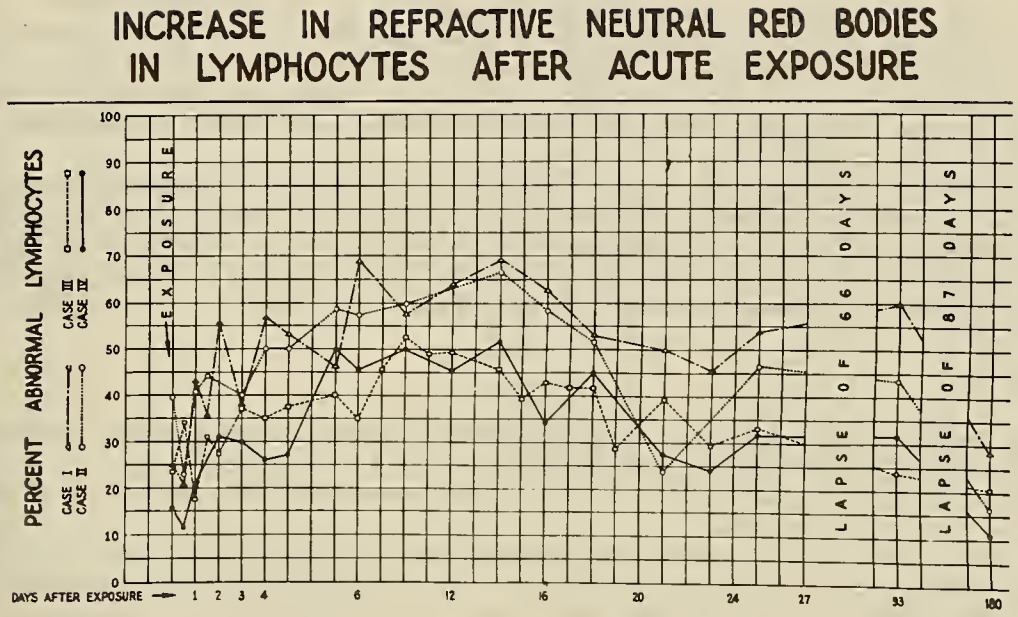

Figure 9. Chart showing the change in per cent abnormal lymphocytes of four persons exposed simultaneously to a burst of ionizing radiation. Only cases I and II sustained enough damage to blood-forming tissues to affect the blood count quantitatively.

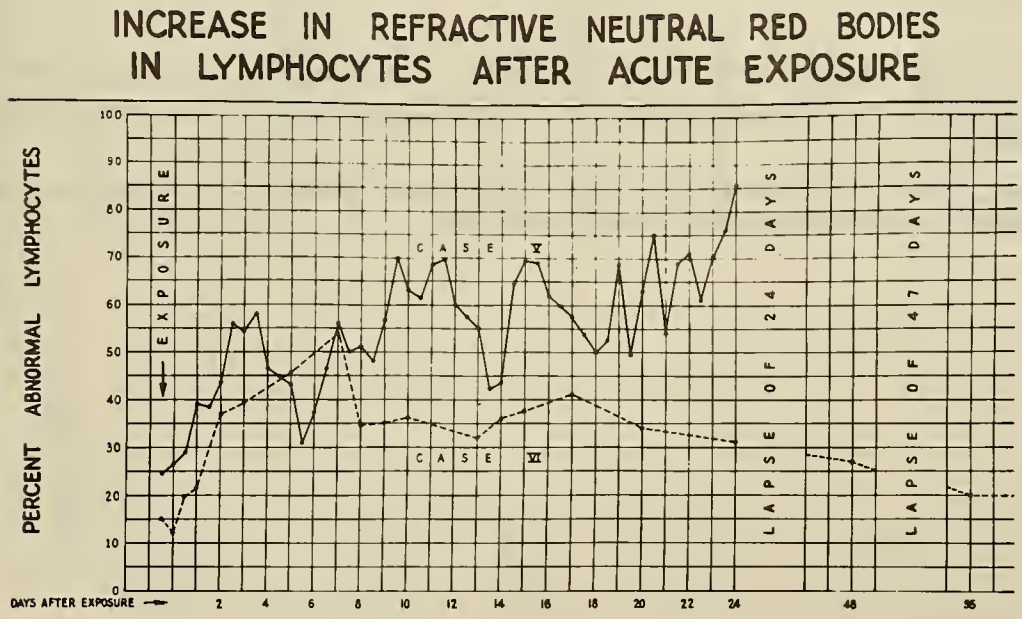

Figure 10. Chart showing the change in per cent abnormal lymphocytes of two persons exposed to a single dose of ionizing radiation. Case V died on the twenty-fourth day following exposure.

pointing to the fact that exposure to toxic chemicals changes the per cent and absolute number of abnormal cells since nine of the fourteen points above the 20 per cent abnormal cell level represent plumbers who are frequently exposed to lead fumes. These subjects have shown no clinical or laboratory evidence of plumbism except for an occasional mild degree of basophilic stipling of the red blood cells. An increase in abnormal lymphocytes is also found in persons working with industrial nonradioactive chemicals besides uranium and lead.

It is evident from Figures 9 and 10 that exposure to a single large dose of ionizing radiation increases the proportion of abnormal cells in the circulating blood. The response of the lymphocytes of the four subjects in Figure 9 is essentially the same although the radiation dosage of the subjects differed by a factor of 10. It must be concluded, therefore, that the increase in abnormal lymphocytes is not proportional to the dosage when administered as a single brief exposure.

SUMMARY

1. Analysis of the total leucocyte counts of persons chronically exposed to ionizing radiation and toxic chemicals shows a significant statistical decrease in the exposed groups.

2. Analysis of the absolute number and per cent lymphocytes in the differential counts of the same persons show no significant change.

3. Morphologic study of supravital preparations of blood cells of persons chronically and acutely exposed to ionizing radiation indicates a striking increase in the number of refractive neutral red bodies in the cytoplasm of the circulating lymphocytes. An increase in neutral red bodies is also found in persons working with toxic chemicals.

4. It is shown that these neutral red bodies have high density and may be considered to be granules. They have not been identified in fixed preparations.

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2. Prigosen, R.E., "Vital Staining of Tumor Cells After Roentgen Rays," J. Cancer Research 8:305 (1924).

3. Schrek, Robert, "Dark-field Observations on Lymphocytes Exposed to X-Rays and Other Injurious Agents," Proc. Soc. Exper. Biol. & Med. 64:381 (1947).

Chart I - Type and Degree of Exposure of Work Groups

Group	Number of Persons in Group	Description of Work	Type and Degree of Exposure
Group A (Unexposed)			
Class I			
Military personnel	62	Physical work out of doors	None
Civilian personnel	40	Indoor work ranging from clerical and administrative jobs to theoretician	None
Ambulatory clinic patients with upper respiratory infection	8	Varied	None
Class II			
Laboratory technicians and scientists	10	Laboratory work	None, but incidental exposure to radiation cannot be excluded completely
Skilled craftsmen	49	Plumbing, carpentry, electrical work and machine shop work	No known exposure to radiation. Plumbers exposed to lead oxide fumes within toxic limits; machinists exposed to metal fumes
Group B. External radiation.			
Class I	25	Laboratory work	Incidental exposure to external radiation; average exposure less than 0.3 - 0.5 r per month
Class II	41	Laboratory work	Intermittent exposure chiefly to natural sources; average exposure 1 r per month or less
Class III	25	Laboratory work	Consistent exposure chiefly to accelerating equipment (cyclotron, Van der Graf, etc.); average exposure 2.0 r or less; rarely did dose in any given day approach 0.1 r

Chart I (continued).

Group	Number of Persons in Group	Description of Work	Type and Degree of Exposure
Class IV	22	Laboratory work	Consistent exposure over six months period to strong radioactive sources; average monthly exposure 3.0 r or less, but daily exposures frequently exceeded 0.1 r and sometimes reached 0.5 r
Group C. Radioactive Material A.			
Class I	8	Laboratory work	Contact with radioactive material A, an alpha particle emitter; no deposition of material within body according to reasonably sensitive tests
Class II	17	Laboratory work	Contact with radioactive material A; some deposition of material in the body but average amount below maximum permissible (tolerance) value
Radioactive Material B.			
Class I	29	Laboratory work	Contact with radioactive material B, an alpha particle emitter; no deposition of material in the body according to moderately sensitive test
Class II	6	Laboratory work	Contact with radioactive material B; deposition of material in the body approaching maximum permissible amounts
Group D			
Uranium	20	Laboratory and plant work	Mild to moderate exposure to uranium chiefly as oxide fumes

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